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# Nosocomial multidrug-resistant *Acinetobacter baumannii* in the neonatal intensive care unit in Gaza City, Palestine

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## KEYWORDS

Nosocomial infection;  
Multi-drug resistance;  
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unit;  
*Acinetobacter baumannii*

## Summary

**Objectives:** We performed a prospective case–control study of bloodstream infections in order to determine the infection rate of *Acinetobacter baumannii* and to determine the risk factors associated with infection and mortality.

**Methods:** Between February 2004 and January 2005, 579 consecutive blood specimens were collected from the two neonatal intensive care units (NICUs) of Al-Nasser and Al-Shifa hospitals in Gaza City.

**Results:** Forty (6.9%) isolates of *A. baumannii* were obtained from neonates aged under 28 days. Of the patients, 62.5% were male and 37.5% were female. Compared to matched, uninfected controls, statistically significant risk factors were weight <1500 g (odds ratio (OR) 3.89,  $p < 0.001$ ), age <7 days (OR 2.33,  $p = 0.027$ ), median hospitalization of =20 days (OR 3.1,  $p = 0.003$ ), mechanical ventilation (OR 3.5,  $p = 0.001$ ), use of a central venous catheter (CVC; OR 10.5,  $p < 0.001$ ), and prior antibiotic use (OR 4.85,  $p = 0.003$ ). The overall mortality was also significantly different (overall mortality 37.5% in cases vs. 12% in uninfected controls; OR 4.4,  $p = 0.001$ ). Compared to infected controls, statistically significant risk factors were mechanical ventilation (OR 2.68,  $p = 0.008$ ), use of a CVC (OR 6.68,  $p < 0.001$ ), and prior antibiotic use (OR 5.68,  $p = 0.001$ ). The multidrug-resistant type was significantly associated with death in the neonates ( $p = 0.023$ ). The isolates of *A. baumannii* were resistant to commonly used antibiotics, while susceptible to meropenem (92.5%), imipenem (90%), ciprofloxacin (75%), gentamicin (57.5%), and ceftriaxone (50%).

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**Conclusions:** The infection rate with multidrug-resistant *A. baumannii* is considerable and alarming in NICU infants, and is associated with significant mortality.

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## Introduction

Newborns receiving care in neonatal intensive care units (NICUs) are at an increased risk of nosocomial infections because of immaturity of the immune system and barrier functions of the skin and gastrointestinal tract, and the invasive diagnostic and therapeutic procedures they undergo.<sup>1</sup> NICU patients are at high risk of nosocomial bloodstream infections (NBSI) because of the high acuity care, prolonged hospitalization, and frequent invasive procedures. Previous studies have found the incidence of NBSI in NICUs to vary between 5% and 32%.<sup>2</sup>

Multidrug-resistant (MDR) *Acinetobacter baumannii* has recently been established as a leading nosocomial pathogen in several hospitals.<sup>3,4</sup> With the spread of multi-resistant bacteria, the treatment of NBSI has become a challenging task.<sup>5</sup> Hospital outbreaks have been described from various geographic areas,<sup>6,7</sup> and this organism has become endemic in some of them. Villegas and Hartstein reviewed *Acinetobacter* outbreaks occurring from 1977 to 2000 and hypothesized that endemicity, increasing rate, and increasing new resistance to antimicrobial drugs in a collection of isolates suggest transmission.<sup>8</sup>

Almost 28 years ago, researchers observed acquired resistance of *A. baumannii* to antimicrobial drugs commonly used at that time, among them aminopenicillins, ureidopenicillins, first- and second-generation cephalosporins, cephamycins, most aminoglycosides, chloramphenicol, and tetracyclines.<sup>9</sup>

In the current study we sought to determine the prevalence of *A. baumannii* infection in NICU patients, and to assess the characteristics and risk factors for MDR *A. baumannii* acquisition during hospitalization.

## Materials and methods

### Clinical setting

This study was conducted in the NICUs of Al-Shifa and Al-Nasser pediatric hospitals. The NICU at Al-Shifa Hospital has a capacity of 10 beds, while that of Al-Nasser Paediatric Hospital has a capacity of eight beds, reserved for premature neonates and neonates requiring intensive care such as mechanical ventilation and cardiac support.

### Design of the study

This was a case–control study conducted from February 2004 to January 2005. A consent form was signed by parents of the neonates in compliance with the Helsinki Declaration and the Palestinian Ministry of Health. Clinical data, including information on demographic characteristics, length of hospital stay, presence of catheters, invasive diagnostic and therapeutic procedures, parenteral nutrition, laboratory examinations, antibiotic usage, Apgar score at 1 minute, and mechanical ventilation were collected.

## Definitions

Sepsis cases were defined as those neonates hospitalized in the NICU who developed *A. baumannii* infection after 48 h of admission to the NICU, confirmed by laboratory examinations.

Uninfected controls were those neonates hospitalized during the same time period as the sepsis cases, matched for age and birth weight, with no diagnosis of bloodstream infection. Infected controls were those neonates who had acquired a bloodstream infection during hospitalization, caused by organisms other than *A. baumannii*. The uninfected and infected controls were used to identify risk factors, presenting signs and symptoms, and outcomes specific to *A. baumannii* that may facilitate and expedite diagnosis and intervention.

## Microbiological analysis

Nursing staff obtained venous blood from NICU newborns by means of aseptic techniques. Briefly 0.2 ml of blood were drawn into bottles containing 10 ml of supplemented trypticase soy broth (Roche) and incubated at 37 °C. After 24 h, blood cultures were subcultured conventionally on plates of sheep blood agar, Chocolate agar, and MacConkey agar (Difco) with aerobic incubation at 37 °C for 18–24 h. Where the blood cultures were negative after incubation for 24 h, inoculation into plates as mentioned above was repeated over the course of a week. Isolates were identified as *A. baumannii* by a negative oxidase test, catalase positive reactions, and Analytical Profile Index (API 20NE) system updated profile (Biomerieux SA, France). Other Gram-negative organisms were identified by API 20E and API 20NE, while Gram-positive staphylococci and streptococci were identified by culture characteristics, catalase, coagulase activities, API 20 Staph, and API 20 Strep.

Multiple blood cultures yielding the same organism from the same patient were considered to relate to a single infection. The following variables were recorded: age, sex, birth weight, and gestational age, and information on intensive care procedures and treatment of bloodstream infection, based on the definitions of the Centers for Disease Control and Prevention (CDC).<sup>10</sup>

The antibiotic susceptibilities of *A. baumannii* isolates were determined by the disk diffusion method on Mueller–Hinton agar plates, using calibrated inoculum of the isolates based on McFarland standard, with the following antibiotics: piperacillin, trimethoprim–sulfamethoxazole (SXT), cefotaxime, ceftriaxone, gentamicin, amikacin, ceftazidime, ciprofloxacin, imipenem, and meropenem.<sup>11</sup>

We defined *A. baumannii* as MDR when the organism was resistant to three antibiotics out of four of the following: ceftazidime, ciprofloxacin, gentamicin, and imipenem.<sup>12</sup>

## Data analysis

Data were analyzed using SPSS version 13 (SPSS, Inc., Chicago, IL, USA). Potential risk factors were assessed by

calculating the odds ratio (OR). Categorical variables were analyzed using Pearson's Chi-square or Fisher's exact test. All tests were two-tailed with  $p < 0.05$  considered significant.

## Results

### Patient characteristics

During the period February 2004 to January 2005, blood specimens from 579 neonates of Al-Nasser and Al-Shifa hospital NICUs in Gaza City were collected for isolation of *A. baumannii*. During this period, 40 cases of *A. baumannii* infection were reported from neonates aged less than 28 days; the incidence was 6.9%. Of the cases, 92.5% were recorded from Al-Shifa Hospital and 7.5% from Al-Nasser NICU. Case patients were similar to their matched control patients with respect to mean age (under 28 days); the patient group had a median age of 8.0 days, while the control group had a median age of 10.0 days. The distribution of cases during the study period (monthly) showed the highest occurrence during the months of April, July, and September.

Univariate analysis of the study patient characteristics of those neonates that survived and those that died was undertaken to assess the prognostic factors for *Acinetobacter* infection and their association with mortality (Table 1).

Inappropriate antibiotic treatment and multi-resistant type were significantly associated with death: 22.2% (8/36) and 22.5% (9/40) of the patients, respectively. In addition, there was an association between leukopenia and mortality (27.5%). No statistical significance was found with regard to age, sex, hospital stay, maternal status, and other clinical and laboratory signs (hypothermia, jaundice, anemia, thrombocytopenia).

### Organisms

In the current study, the pathogens other than *A. baumannii* isolated among bacteremic cases were *Escherichia coli* (27, 5.0%), *Staphylococcus aureus* (17, 3.1%), *Klebsiella spp* (16, 3.0%), coagulase-negative staphylococci (15, 2.8%), *Enterobacter spp* (9, 1.7%), *Streptococcus spp* (8, 1.5%), *Citrobacter spp* (7, 1.3%), and *Pseudomonas aeruginosa* (7, 1.3%).

### Risk factors

Risk factors and outcome (death) associated with cases of *A. baumannii* sepsis versus uninfected controls are shown in Table 2. Case patients were significantly more likely to have received mechanical ventilation (OR 3.5), had prior use of antibiotics (OR 4.85), and had a central venous catheter (CVC; OR 10.5). In addition case patients were more likely to have had a birth weight of <1500 g (OR 3.89), age <7 days (OR 2.33), and a median of hospitalization of = 20 days (OR 3.1). Several other variables had a tendency to be more associated with case patients, but the values did not reach statistical significance, such as weeks of gestation, sex, and Apgar score at 1 minute <7. The overall crude mortality rate was 15/40 (37.5%), with an OR of 4.4.

Similarly, the risk factors and outcome (sepsis-related death) associated with cases of *A. baumannii* sepsis versus matched infected controls are displayed in Table 3. Factors

**Table 1** Demographic and clinical characteristics of neonates infected with *Acinetobacter baumannii* (N = 40)

| Characteristics                    | Neonates that survived (n = 25) | Neonates that died (n = 15) | p-Value           |
|------------------------------------|---------------------------------|-----------------------------|-------------------|
| Age                                |                                 |                             |                   |
| ≥7 days                            | 15 (60)                         | 6 (40)                      | 0.22              |
| <7 days                            | 10 (40)                         | 9 (60)                      |                   |
| Sex                                |                                 |                             |                   |
| Male                               | 15 (60)                         | 10 (66.7)                   | 0.67              |
| Female                             | 10 (40)                         | 5 (33.3)                    |                   |
| Antibiotype                        |                                 |                             |                   |
| Multi-resistant                    | 6 (24)                          | 9 (60)                      | 0.023             |
| Susceptible                        | 19 (76)                         | 6 (40)                      |                   |
| Antibiotic treatment               |                                 |                             |                   |
| Appropriate                        | 19 (76)                         | 3 (20)                      | 0.01 <sup>a</sup> |
| Inappropriate                      | 6 (24)                          | 8 (53.3)                    |                   |
| No antibiotics                     | -                               | 4 (26.7)                    |                   |
| Clinical and laboratory signs      |                                 |                             |                   |
| Hypothermia                        | 6 (24)                          | 8 (53.3)                    | 0.06              |
| Jaundice                           | 8 (32)                          | 4 (26.7)                    | 1.0 <sup>a</sup>  |
| Anemia                             | 9 (36)                          | 10 (66.7)                   | 0.06              |
| Thrombocytopenia                   | 12 (48)                         | 9 (60)                      | 0.46              |
| Leukopenia                         | 8 (32)                          | 11 (73.3)                   | 0.011             |
| Median of hospitalization          |                                 |                             |                   |
| 20 days                            | 15 (60)                         | 10 (66.7)                   | 0.67              |
| 12 days                            | 2 (8)                           | 3 (20)                      | 0.34 <sup>a</sup> |
| 8 days                             | 8 (32)                          | 2 (13.3)                    | 0.27 <sup>a</sup> |
| Maternal status                    |                                 |                             |                   |
| Hypertension                       | 9 (36)                          | 7 (46.7)                    | 0.5               |
| Diabetes mellitus                  | 5 (20)                          | 2 (13.3)                    | 0.69              |
| Urinary tract infection            | 10 (40)                         | 7 (46.7)                    | 0.68              |
| Premature rupture of the membranes | 1 (4)                           | 3 (20)                      | 0.14 <sup>a</sup> |

Results are n (%).

<sup>a</sup> Fisher's exact test.

significantly associated with cases were prior use of antibiotics (OR 5.68,  $p = 0.001$ ), use of a CVC (OR 6.68,  $p < 0.001$ ), mechanical ventilation (OR 2.68,  $p = 0.008$ ), and sepsis-related death (OR 3.94,  $p = 0.001$ ). Other factors also appeared to be more associated with sepsis cases, but the values did not reach statistical significance.

### Antibiotic susceptibility

The antibiotic susceptibility pattern showed 92.5% of the isolates were susceptible to meropenem, 90% to imipenem, 75% to ciprofloxacin, 57.5% to gentamicin, and 50% to

**Table 2** Bivariate risk factors and outcome associated with cases of *Acinetobacter baumannii* sepsis versus uninfected controls

| Risk factors                           | Cases (n = 40) | Uninfected controls (n = 100) | OR   | p-Value           |
|----------------------------------------|----------------|-------------------------------|------|-------------------|
| Weight at birth                        |                |                               |      |                   |
| ≥1500 g                                | 15 (37.5)      | 70 (70)                       | 3.89 | 0.001             |
| <1500 g                                | 25 (62.5)      | 30 (30)                       |      |                   |
| Sex                                    |                |                               |      |                   |
| Female                                 | 15 (37.5)      | 45 (45)                       | 1.36 | 0.42              |
| Male                                   | 25 (62.5)      | 55 (55)                       |      |                   |
| Age                                    |                |                               |      |                   |
| ≥7 days                                | 21 (52.5)      | 72 (72)                       | 2.33 | 0.027             |
| <7 days                                | 19 (47.5)      | 28 (28)                       |      |                   |
| Weeks of gestation                     |                |                               |      |                   |
| 26–29                                  | 5 (12.5)       | 15 (15)                       | 0.81 | 0.70              |
| 30–33                                  | 10 (25)        | 25 (25)                       | 1.0  | 1.0               |
| 34–36                                  | 25 (62.5)      | 60 (60)                       | 1.11 | 0.78              |
| Median of hospitalization              |                |                               |      |                   |
| =20 days                               | 25 (62.5)      | 35 (35)                       | 3.1  | 0.003             |
| =12 days                               | 5 (12.5)       | 23 (23)                       | 0.48 | 0.16              |
| =8 days                                | 10 (25)        | 42 (42)                       | 0.46 | 0.06              |
| Mode of delivery                       |                |                               |      |                   |
| Vaginal                                | 28 (70)        | 67 (67)                       | 1.15 | 0.73              |
| Cesarean                               | 12 (30)        | 33 (33)                       |      |                   |
| Premature rupture of the membranes     | 4 (10)         | 13 (13)                       | 0.74 | 0.78 <sup>a</sup> |
| Apgar score at 1 minute                |                |                               |      |                   |
| ≥7                                     | 21 (52.5)      | 67 (67)                       | 1.8  | 0.11              |
| <7                                     | 19 (47.5)      | 33 (33)                       |      |                   |
| Prior antibiotic use                   | 36 (90)        | 65 (65)                       | 4.85 | 0.003             |
| Utilization of central venous catheter | 29 (72.5)      | 20 (20)                       | 10.5 | 0.001             |
| Mechanical ventilation                 | 24 (60)        | 30 (30)                       | 3.5  | 0.001             |
| Clinical outcome                       |                |                               |      |                   |
| Death                                  | 15 (37.5)      | 12 (12)                       | 4.4  | 0.001             |

Results are n (%).

<sup>a</sup> Fisher's exact test.

ceftriaxone, whereas isolate susceptibilities to amikacin, cefotaxime, ceftazidime, SXT, and piperacillin were 37.5%, 35%, 30%, 22.5%, and 10%, respectively. Despite a small number of bacterial strains being resistant to imipenem and meropenem, 15 strains from both hospitals were considered MDR, which constituted 37.5% of the total isolated strains.

## Discussion

*Acinetobacter* species have emerged as important nosocomial pathogens that are often MDR and associated with life-threatening infections.<sup>13</sup> *A. baumannii*, a clinically important species has a tendency toward cross-transmission, particularly in ICUs, where numerous outbreaks have been encountered.<sup>14</sup>

To the best of our knowledge, this study is the first to focus on septicemia in NICUs in Gaza City. The present study was conducted to determine the prevalence of *A. baumannii* as well as to identify risk factors for acquisition of MDR *A. baumannii* present in the NICU.

The prevalence rate in this study was 6.9% of the total bloodstream samples (579) collected in the NICU over a 1-year period, with 37.5% death due to *A. baumannii*.

Several risk factors showed an association with the development of *A. baumannii* infection and sepsis-related death. The most predominant risk factors with a statistically significant association were submission to invasive procedures, mechanical ventilation, utilization of a CVC, and the presence of conditions such as very low birth weight. These same risk factors have been described previously.<sup>3,15</sup> *A. baumannii* sepsis in the NICU was associated with a significant risk of

**Table 3** Bivariate risk factors and outcome associated with cases of *Acinetobacter baumannii* sepsis versus infected controls

| Risk factors                           | Cases (n = 40) | Infected controls (n = 106) | OR   | p-Value          |
|----------------------------------------|----------------|-----------------------------|------|------------------|
| Weight at birth                        |                |                             |      |                  |
| ≥1500 g                                | 15 (37.5)      | 45 (42.5)                   |      |                  |
| <1500 g                                | 25 (62.5)      | 61 (57.5)                   | 1.23 | 0.59             |
| Sex                                    |                |                             |      |                  |
| Female                                 | 15 (37.5)      | 43 (40.6)                   |      |                  |
| Male                                   | 25 (62.5)      | 63 (59.4)                   | 1.14 | 0.74             |
| Age                                    |                |                             |      |                  |
| ≥7 days                                | 21 (52.5)      | 68 (64.2)                   |      |                  |
| <7 days                                | 19 (47.5)      | 38 (35.8)                   | 1.62 | 0.2              |
| Weeks of gestation                     |                |                             |      |                  |
| 26–29                                  | 5 (12.5)       | 28 (26.4)                   | 0.4  | 0.073            |
| 30–33                                  | 10 (25)        | 23 (21.7)                   | 1.2  | 0.67             |
| 34–36                                  | 25 (62.5)      | 55 (51.9)                   | 1.5  | 0.25             |
| Median of hospitalization              |                |                             |      |                  |
| =20 days                               | 25 (62.5)      | 48 (45.3)                   | 2.0  | 0.064            |
| =12 days                               | 5 (12.5)       | 26 (24.5)                   | 0.44 | 0.11             |
| =8 days                                | 10 (25)        | 32 (30.2)                   | 0.77 | 0.54             |
| Mode of delivery                       |                |                             |      |                  |
| Vaginal                                | 28 (70)        | 76 (71.7)                   | 0.92 | 0.84             |
| Cesarean                               | 12 (30)        | 30 (28.3)                   |      |                  |
| Premature rupture of the membranes     | 4 (10)         | 13 (12.3)                   | 0.79 | 1.0 <sup>a</sup> |
| Apgar score at 1 minute                |                |                             |      |                  |
| ≥7                                     | 21 (52.5)      | 62 (58.5)                   |      |                  |
| <7                                     | 19 (47.5)      | 44 (41.5)                   | 1.27 | 0.51             |
| Prior antibiotic use                   | 36 (90)        | 65 (61.3)                   | 5.68 | 0.001            |
| Utilization of central venous catheter | 29 (72.5)      | 30 (28.3)                   | 6.68 | 0.001            |
| Mechanical ventilation                 | 24 (60)        | 38 (35.8)                   | 2.68 | 0.008            |
| Clinical outcome                       |                |                             |      |                  |
| Sepsis-related death                   | 15 (37.5)      | 14 (13.2)                   | 3.94 | 0.001            |

Results are n (%).

<sup>a</sup> Fisher's exact test.

mortality. This circumstance is not explained by differences in infection control measures, but rather it could be attributed to the occurrence of MDR strains. Inappropriate therapy was significantly associated with death in 22.2% (8/36) of neonates. The study also revealed that MDR *A. baumannii* type and leukopenia were significantly associated with mortality. These findings are consistent with other recent studies.<sup>16,17</sup>

In vitro susceptibility tests showed that meropenem and imipenem were the most effective drugs against *A. baumannii* isolates in Palestine, while susceptibility to third- or fourth-generation cephalosporins and ciprofloxacin varied considerably. Although ciprofloxacin is not recommended in the treatment of neonates, it was used because of the lack of an alternative in a life-threatening situation.

The high MDR *A. baumannii* rate (37.5%) found in this study may be associated with the high frequency at which these antimicrobial drugs were used for both the prophylactic and therapeutic treatment of hospitalized newborns. This practice may have exerted selective pressures leading to the emergence of MDR strains,<sup>18,19</sup> which in turn may have stimulated the acquisition of genes encoding resistance mechanisms via horizontal transfer mechanisms between environments. It is of particular interest that in this study a significant number of patients received antimicrobial drugs before positive blood culture presentation (empiric use), due to clinical manifestations suggestive of a bloodstream infection. A progressive decrease in the effectiveness of third-generation cephalosporins against *Acinetobacter* has been



coupled with the increased use of these antibiotics.<sup>13,20</sup> We re-emphasize that intravenous broad-spectrum antibiotics should be used with caution. Ceftazidime and/or cefotaxime use should be discontinued in units where resistant strains for these two antibiotics are being increasingly reported. With regard to ceftazidime- and cefotaxime-resistant strains in our study, the hospital ICU was advised to use other antibiotic combinations like an effective beta-lactam or carbapenem (imipenem) along with amikacin. The combined use of various antibiotics would be judicious.

In conclusion, the MDR *A. baumannii* infection rate is considerable and alarming in NICU infants, and is associated with significant mortality. Risk factors include low birth weight, the use of CVCs, mechanical ventilation, and prior antibiotic use.

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